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Discovery of small molecule inhibitors targeting the SUMO-SIM interaction using a protein interface consensus approach

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The SUMO-SIM is a challenging protein-protein interaction drug target. We present a virtual screening approach incorporating the consensus of protein interactions that led to the discovery of non-peptidic inhibitors. The most potent inhibitors have low micromolar potency and the binding affinity and interface was validated using multiple assays and HSQC-NMR.

Sumoylation is a post-translational modification of proteins that regulates important cellular functions, such as cell proliferation, chromosome packing and dynamics, DNA replication and repair, genome integrity, nuclear transport and signal transduction.[1-7] Sumoylation of target proteins consists of the covalent attachment of one of the Small Ubiquitin-like MOdifier (SUMO) proteins to a lysine located in a consensus sequence of the target proteins by means of an enzymatic cascade involving several enzymes: the E1 activating enzyme (AOS1/UBA2), the E2 conjugating enzyme (UBC9) and sometimes an E3 ligase, which serves as a matchmaking protein.[8] Emerging evidence suggests that sumoylation plays a general role in regulating protein-protein interactions (PPIs).[9] This is mediated via the recognition of SUMO proteins by a SUMO interaction motif (SIM).[10-12] Given its role in many important cellular processes, the sumoylation pathway has also been linked to a significant number of pathogenicities including neurodegenerative diseases and cancer.[1, 4, 13] This makes sumoylation a novel drug target. Since the enzymatic steps of the sumoylation process are all catalysed by a single set of enzymes for the different sumoylation targets, the PPIs that drive the specificity are potentially an important target. There is an interest for the development of druglike molecules inhibiting the SUMO-SIM interaction to study the poorly understood role of PPIs in the sumoylation process as well as potential therapeutic applications. The pharmaceutical relevance of this PPI towards chemo- and radio-sensitization of DNA damage sensitive cancer cells has been demonstrated by its inhibition using an overexpressed SIM peptide or a gold nanoparticle linked peptide-like inhibitor.[14, 15] With the exception of peptide or antibody like inhibitors,[16] druglike small molecule inhibitors have not yet been reported. Traditionally PPIs have been considered undruggable, but in recent years different Small Molecule PPI Inhibitors (SMPPIIs) binding to druggable interaction sites have been reported resulting in an increased interest.[17] Here we report the discovery of SMPPIIs targeting the SUMO-SIM interaction using an interaction consensus based approach.

Our method consisted of a funnel approach, in which different rational methods were combined to screen a commercial library *in silico*, sieving out at every step molecules unlikely to be active for the desired target. A more detailed description is given in ESI[†]. Structural analysis of the interface indicated the absence of a druglike cleft, and while small molecule binding site detection algorithms such as HotPatch[18] were unable to identify a suitable interface, abundant structural information indicates the possibility for peptides and proteins to bind to this interface. Electrostatic analysis of the SUMO-SIM interface indicated a positively charged environment. To enforce the complementarity with receptor, the screening database was filtered for compounds with a total negative charge or more hydrogen bond acceptor than donor atoms. The collected SUMO-SIM structures were superposed and the common interactions at the superficial interaction interface were clustered and transformed into a pharmacophore query, thereby effectively capturing the essential elements of molecular recognition at the shallow and flexible PPI interface. The pharmacophore query represented the key interactions of molecular recognition consisting of 3 hydrophobic areas, and beta-strand hydrogen bonding pattern with the SUMO backbone, valid for every conformation of the binding interface as retrieved from the PDB.[19, 20] This pharmacophore model was then used to query the filtered conformational database (Figure 1) using the pharmacophore search implementation of the MOE (Molecular Operating Environment, Chemical Computing Group, Montreal, Canada).

The retained molecules were subsequently docked into two different SUMO interface conformations (PDB 1WYW and 1Z5S). The docked conformations were post-filtered using the pharmacophore query, pairwise similarity of binding modes between the two SUMO interface conformations and electrostatic similarity with the DAXX peptide, a transcriptional co-repressor containing a SIM.[12] Hence, all compounds that were peptide-like, adopted a binding mode different from the key interactions described by the pharmacophore query, exhibited a dissimilar binding mode in the two docking receptors,

and dissimilar from the electrostatic potential field of the DAXX peptide have been removed.[21]

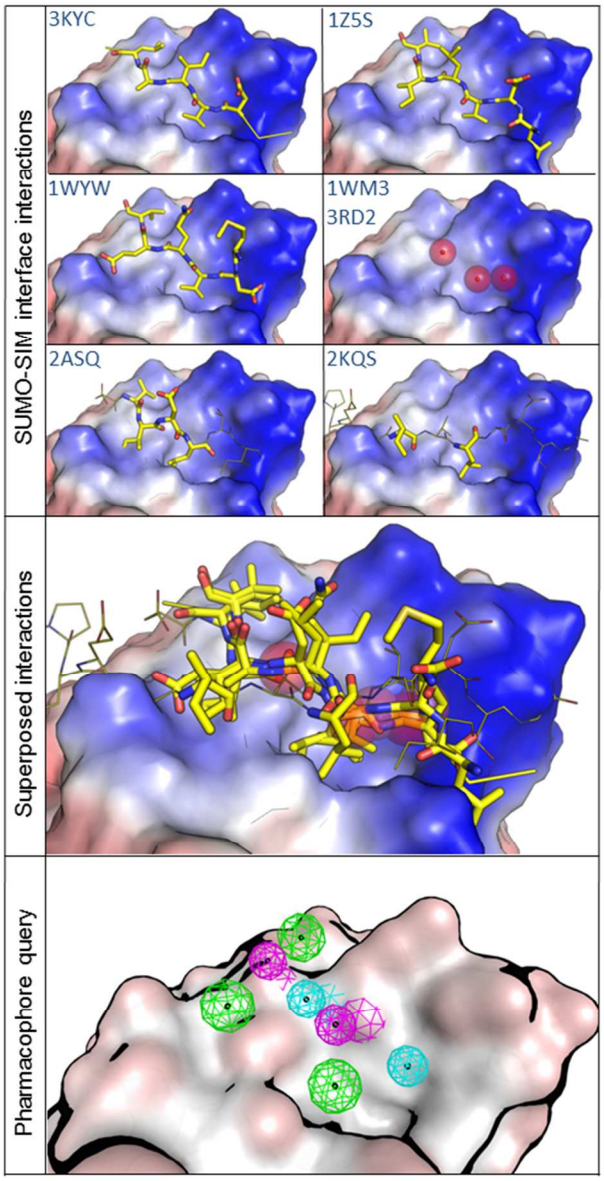


Figure 1: To identify the interaction consensus and represent it as a pharmacophore query, 5 different structures with SUMO bound to an SIM and 2 with conserved water molecules (top panel) were superimposed (middle panel) and the common interactions were represented as a pharmacophore (bottom panel). This pharmacophore was used to query a conformational database and subsequently post-filter the docking results.

After rational analysis of the remaining compounds,[22] a final selection of 64 compounds was made. These compounds were acquired from Namiki Shoji and tested using the AlphaLISA bioluminescent assay to inhibit the interaction between the SUMO1 and SIM at 500μM (see ESI†). To rule out false positive compounds, the TruHits assay was performed, which discarded one case of a potential false positive compound. The AlphaLISA assay identified 11 hit compounds that inhibited the SUMO-SIM interaction more than 50% (see ESI†). Thus, our virtual screening approach has a hit-rate of ±15% for this challenging target. Following a SAR by catalogue approach to identify stronger derivatives, 79 analog molecules were ordered from the same vendor and tested at lower concentrations (100 and 30 μM) together with the initial hit compounds. All these inhibitors fulfil the pharmacophore query, which describes the common interactions at the PPI interface for the different SUMO-SIM structures.

Interestingly, 4 PPI stimulators could be identified in addition to the 33 inhibitory compounds (see ESI†). These 33 inhibitory compounds can be clustered into 6 different classes, while the 4 PPI stimulators belong to the same chemotype. A summary of these compounds is shown in Table S1. Although stimulators are uninteresting for our current research, they indicate the possibility of PPI-stimulation and together with recent reports[23, 24] may provide an interesting type of drug candidate in the future. For further validation of the most potent SMPPII hit compounds, a Surface Plasmon Resonance (SPR) assay was employed (Table S1). The IC_{50} and the K_D values for the most potent compounds (SSI-091, SSI-096, SSI-104) are in the low micromolar range in both AlphaLISA and SPR, and are valid hit compounds for further optimization (Table 1 and ESI†). The affinities of these hit compounds for the SUMO2/3 protein were also measured using the SPR assay. The affinities (K_D 1.9±1.3 μM for SSI-091, K_D 59.0±1.0 μM for SSI-096 and K_D 29.0±4.0 μM for SSI-104) are almost identical as that for the SUMO1 protein. The key interactions of these compounds are made with the protein backbone, which is identical in different SUMO isoforms explaining the similar potency. SSI-091 appears to be most potent compound of the three. This is most likely because of the higher polarity of the compound which is in agreement with the very polar binding surface of the SUMO proteins, enhancing the molecular recognition of the compound.

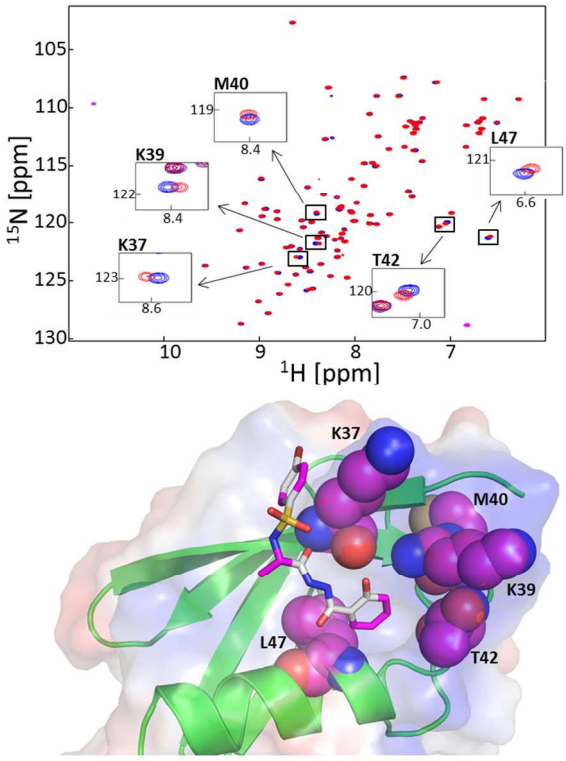


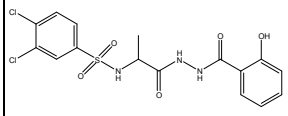
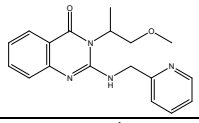
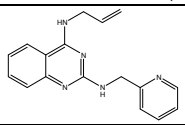
Figure 2: The ^{1}H,^{15}N HSQC spectra of ^{13}C,^{15}N labelled SUMO1 (blue) and ^{13}C,^{15}N labelled SUMO1 with SSI-091 (red) are shown.

Residues with large changed chemical shifts are labelled, boxed and expanded views are also shown (top). At the bottom, the predicted conformation using docking simulation with the pharmacophore postfilter is shown to be in full agreement with the NMR experiments and the consensus interaction pharmacophore model. SUMO1 is visualized as a green cartoon. The NMR-determined contacting residues are represented as pink spheres. The inhibitor is visible as sticks. Pink atoms agree with the contacting carbon atoms identified from the STD experiment.

To further characterize the binding of the most potent inhibitor (SSI-091) to SUMO1, NMR experiments were carried out (see ESI†). The NMR STD experiments indicated a direct interaction of the SSI-091 with the SUMO1 protein. Following NMR $^{1}H,^{15}N$-HSQC experiments, chemical shifts were observed revealing the interacting amino acids on the SUMO1 protein. The observed shifts as well as the compound atom epitope mapping agree with the modelled binding mode according to the pharmacophore query and docking simulations from the virtual screening experiment (Figure 2), which again demonstrates the validity of the pharmacophore based approach to identify the key interactions at the PPI interface. Attempts to analyse the effect of these compounds *in cellullo* using a split-luciferase complementation system[25] failed due to compound toxicity (data not shown).

Our research demonstrates the feasibility to target the SUMO-SIM interaction with druglike compounds for PPI inhibition. In the future, optimized derivatives or novel compounds identified using a similar strategy may prove to be useful chemical probes to study the SUMO-SIM interaction or chemo- and radiation sensitizing pharmaceutical agents.

Table1: The most potent inhibitors and their potency in AlphaLISA and SPR experiments.

ID-number	Structure	AlphaLISA $IC_{50}(\mu M)$	SPR assay $K_D(\mu M)$
SSI-091		7.5±2.3	1.8±0.7
SSI-096		3.2±0.7	44.5±16.5
SSI-104		21.6±2.0	34.0±2.0

Conclusions

Although the SUMO-SIM interaction can be considered as a challenging target due to the absence of a clear binding pocket, we were able to identify and validate SMPPIIs with low micromolar activity using a PPI interaction consensus based pharmacophore query. A similar *in silico* screening approach that exploits common motifs for molecular recognition at PPI interfaces could thus also be useful for the discovery of SMPPIIs targeting PPIs that appear to be undruggable.

Notes and references

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